being due to the eroded umbones, but with young specimens showing umbonal sculpture, it was an easy matter. Simpson says in his Descriptive Catalogue for both species, that the umbonal sculpture was not seen; hence no description. This museum has numerous specimens of young and adult of both species recently collected, and the young show the umbonal sculpture of both species to be composed of about four coarse ridges; in *minor* they are circular and in *villosa* V-shaped looped.

Mr. Frierson, in a letter of November 27, '16, writes that he has discovered how to differentiate the adults of these two species. He calls attention to an additional small muscle scar (cicatricula?) at the upper end of the anterior muscle scar (cicatrix) in *minor.*

I have just found time to go over the specimens in this museum and open them up, and separate them according to Mr. Frierson's discovery, and I am now prepared to give some additional information. In the majority of specimens a glass is required to see the small scar referred to, and then in the majority of specimens the small scar, instead of being separate, is but an extension of the larger one, which makes it still harder to determine. Simpson says, of *minor*, "anterior scars *deep*," and of *villosa* "muscle scars *scarcely impressed.*" In opening a shell, the *deep* scar in *minor* is at once noticeable from the *shallow* one of *villosa*. *Minor* is a heavier, wider, and shorter shell than *villosa*, and the anterior distance from the umbo is shorter in *minor*.

Florida State Museum.

NOTES ON REPRODUCTION AND GROWTH IN CERTAIN VIVIPAROUS MUSSELS OF THE FAMILY SPHAERIIDAE.

BY RALPH J. GILMORE.

The present study was undertaken in an effort to determine the nature of the reproductive process in certain common forms of the family *Sphaeriidae*. For a long time incubation of the young has been known to occur in European forms, but no investigation has been made of related forms from America.

HISTORICAL. Jacobsen (1828) noted the fact that embryos of Cyclas develop in sacs. He observes that "each ovary is composed of a number of small cylindrical sacs or capsules. When impregnated, these sacs increase in bulk and gradually protrude from the abdomen. By this protrusion they are introduced into the gill cavity but still retain connection with the interior membrane of the ovary. These capsules contain the eggs and the young are developed in them. Each one contains but one egg or young one. As soon as the young has reached a certain size, the capsule bursts and the young is ejected into the gill cavity. We find in the gill cavity at one and the same time, capsules and young both large and small." Apparently the only part of Jacobsen's observations which is correct, is the fact that the young develop in sacs. Later authors fail to confirm his work. Oskar Schmidt (1854) investigated the anatomy of *Cyclas calyculata*. Franz Leidig (1855) studied the anatomy of *Cyclas cornea*. Stepanoff (1865) was the first to point out the brood pouch of Cyclas in its true relation. His observations were confirmed in 1885 by Ziegler. However, the work of both of these men was directed mainly toward segmentation and development of the embryo and their observations on the brood pouch were mere casual notes. In one of his plates Ziegler shows a diagrammatic figure including a small portion of a brood pouch. De Bruyne (1898) in a work on phagocytosis figures a brood pouch. Poyarkoff (1910) published a preliminary note on the incubation of embryos of Cyclas. This was followed in 1911 by a paper on the same form by Schereschewsky. Both of these authors gave considerable attention to the cellular structure of the pouch, its origin and function. The only work that has been published on American forms is that of Drew, who in 1894 described the anatomy of *Sphaerium sulcatum*.

MATERIAL. The material for this work was collected during the summer and fall of 1913, from ponds and streams in the neighborhood of Ithaca, N. Y. Two forms were observed, *Calyculina truncata* and *Sphaerium simile*.

Calyculina truncata is one of the smallest of the *Sphaeriidae*. It averages about eight millimeters long, and seven high. The shell is very fragile, rhombic ovate, the posterior part very squarely cut off, the anterior broadly rounded. The surface is smooth and shining with very fine lines of growth. The color is light yellowish green or greenish horn. It occurs in clear fresh-water ponds or the sheltered parts of rivers, usually embedded in soft sticky mud, the siphons protruding just above the surface. Very often it may be seen climbing about on submerged vegetation. The seasons of greatest apparent abundance are the spring and early summer months. It quite frequently occurs in ponds which are dry throughout the greater part of the year.

Sphaerium simile is one of the largest species of the family. An adult specimen may be eighteen millimeters long and thirteen millimeters high. The shell is rather solid, almost equilateral, transversely oval (Fig. 10), the anterior and posterior margins almost equal. The surface is shining, pale green, with coarse growth-lines in young specimens but in older ones it is dull, dark brown to black with only those growth-lines evident which mark the ends of growth periods. It prefers clear, cold streams but may be found in quiet pools of rivers and lakes. It buries itself in soft mud or debris usually an inch or more below the surface. It communicates with the water above through a small hole in the mud. I have no record of this form occurring in places which are not well supplied with water throughout the year.

Methods. During the early part of the work, expanded animals were fixed in hot water or hot mercuric chloride. This method had one great advantage, in that it allowed very litte contraction of organs. But for cell structure the following was found to be better. The animals were prevented from completely closing the shell by a small piece of wood inserted between the valves. In this condition they were placed in cold saturated mercuric chloride with two to three percent of glacial acetic acid. They were kept in the fixer for twenty-four hours. The acidity of the fixer removed nearly all of the calcium salts of the shell. The remainder was removed by one half to one percent of hydrochloric acid in sixty-seven percent alcohol. The entire animal was imbedded in paraffin. Serial sections were cut from six to ten microns in thickness. Delafield's

Haematoxylin and orange G. in ninety-five percent alcohol were used as stains.

In order to establish the relation of the brood pouch to the gill filaments and water spaces a wax model of parts of a gill was constructed from drawings made on an Edinger machine.

Reproductive Organs. The animal is hermaphroditic. The reproductive organs are situated beneath the pericardium and behind the stomach (Figs 1, 2, 3, 4). They consist of a pair of racemose glands, the anterior part of which produce sperm and the posterior eggs. A common genital duct continues backward, opening into the cloacal chamber of the inner gill near the opening of the kidney.

Plate V, Fig. 2, represents the essential parts of an egg follicle. Each follicle is lined with a single layered epithelium supported by a very heavy basement membrane. Eggs develop by the enlargement of certain cells of the lining epithelium. When a developing egg has grown to four or five times the size of the neighboring cells it is pushed out of its position by a pedestal-like growth of the basement membrane. Thus projected into the lumen of the follicle, it continues to develop until mature, when it drops off.

The sperm-producing follicles (Fig. 3) are irregularly spherical and arranged about their common duct like the parts of a raspberry. Each follicle is made up of a mass of sperm mother cells about its outer part and either fully formed or young sperm cells near the center. The center is hollow and communicates with the common sperm duct. This duct (Pl. V, Fig. 3) extends a short distance backward where it receives the product of the egg follicle, continuing from that point to the exterior as a common genital duct.

Regarding maturation and fertilization Stepanoff (1865) observes, "When the egg has reached a certain size it separates more and more from the wall of the basement tissue until it at last becomes free, in the inner part of the follicle and later falls into the outlet of the sex glands. The separation is affected by the increase of the yolk mass and the resulting weight of the egg. Eggs thus fallen into the duct become surrounded by a mass of fully formed sperm, so that, without doubt, fertilization occurs in this place." Schereschewsky says, "Fertilization takes place in the gill chamber." In the majority of the specimens of Calyculina and Sphaerium ripe sperm and eggs were found to occur in the same individual. None of the specimens had eggs in the genital duct.

BREEDING SEASONS. The breeding season probably continues through the greater part of the year. Observations on this point have been very meager. Animals taken in November and December of 1913 were found to contain, in newly formed brood pouches, eggs some of which were unsegmented and others in very early cleavage stages. Considerably over fifty adult specimens have been sectioned. All were found to contain young in several stages of development.

GILLS. Before considering the structure of the brood pouch it will be necessary to look into the structure of the gills. The gills are four in number, an outer and an inner pair. The outer is much smaller than the inner and falls short anteriorly by about a fourth of its length. Each gill has two lamellae. The outer lamella of the outer gill is attached to the mantle; the inner lamella of the outer gill is attached to the outer lamella of the inner gill and the inner lamella of the inner gill is attached to the body. It is the outer lamella of the inner gill which contains the brood pouches.

The lamellae are made up of gill filaments (Figs. 7, 8, and 11). A typical filament may be compared to a rubber tube sharply bent on itself to form a letter Y. Each filament of one lamella is therefore continuous with one of the other lamellae. The open part of the letter Y represents the cloacal chamber. All water which passes between the filaments finds its way into this chamber and from thence to the exterior. In the anterior and posterior parts of the gill the cloacal chamber is very much reduced (Figs. 8 and 11).

Each filament is a hollow tube which in frontal section appears as an irregular ellipse (Figs. 8 and 11). The outer part is made up of a single layer of heavy cells, strengthened by chitinous rods (Fig. 8). The inner part is a single layer of flattened cells forming a very thin membrane. The hollow part of a filament is the blood space. This blood space may be crossed by an irregular loose network of web-like threads. These probably serve to prevent the membrane from collapsing. In the ventral part of the gill, except at the ventralmost part, the blood spaces of the two lamellae are kept separate as is shown (Figs. 5, 7 and 11).

At irregular intervals adjacent filaments are joined by interfilamentary junctions (Figs. 7, 8 and 11). Small ribbon-like bands of fibrous chitin may join several filaments for a short space. These by holding the filaments together give definite shape to the lamellae which would otherwise be a tangle of tubes. Another type of junction (Fig. 8) is made by the direct fusion of the elements of two adjacent filaments. This is the more common form of junction in the dorsal part of the gill. At the most dorsal part the filaments lose their identity entirely and fuse to form large blood spaces.

Between the filaments are water spaces which communicate with the mantle chamber on the outside and the cloacal chamber on the inside. Water is kept flowing from the mantle chamber to the cloacal chamber and the excurrent siphon by cilia. The outer surface of the filaments is covered with short cilia, the sides have a narrow row of longer ones.

Circulation of the Blood. The most important function of the gills is the purification of the blood. Blood leaves the ventricle by two main arterial trunks, the one supplying the anterior and the other the posterior part of the body. These vessels end in blood spaces which have no definite wall. The spaces of the greater part of the body pour their blood into the inner lamella of the inner gill (Fig. 5). Passing first ventrally in this lamella, it turns at the bottom of the gill and comes upward through the outer lamella. In the dorsal part of this lamella the filaments fuse to form a large sinus which becomes the auricle and empties into the ventricle. The outer gill derives its supply of blood from the mantle and such parts of the body as are near by. Blood enters the outer lamella, crosses to the inner lamella and enters the heart by the same channel that carries blood from the inner gill. It should be noted that the brood pouches are admirably located. For they are bathed by blood which has just left the alimentary tract and later received its supply of oxygen.

Brood Pouch. Fig. 8 represents a fully formed brood pouch as seen in frontal section. The pouch has two distinct walls, an outer and an inner. These are direct continuations of the heavier portions of adjacent gill filaments. The outer wall consists of a thin one-celled membrane made of flat expanded cells. This wall is, in every respect, similar to the membranous part of the gill filaments. The inner wall is also made up of a single layer of cells. A part of this wall is similar to the outer wall though the major portion is composed of very thick glandular cells. Between the outer and the inner walls is a blood space. This space is a modification of the spaces of the two filaments to which the two walls are attached (Fig. 11). Numerous web-like cross threads occur in the blood space. These are similar to those which are found in the spaces of typical filaments. They furnish another proof that the two walls are mere modifications of filaments. The brood pouch may contain but one embryo (as in Fig. 8) or it may enclose a number (as in Fig. 11). A pouch may involve two filaments and only two (as in Fig. 8) or it may be constructed from the parts of several. The inner wall of such a pouch is thrown into folds which divide it into communicating chambers. These folds probably represent the contributions of the several filaments.

Just how the pouch originates is still an open question. Stepanoff (1865) and Schereschewsky (1913) believe it to be a modification of gill filaments. In Calyculina and Sphaerium all the available evidence points to such an origin. The wax model shows the pouch to be a modification of ordinary filaments. The same filaments enter into the structure of the pouch throughout its extent.

Poyarkoff (1910) offers this theory for the origin of the pouch: "When the embryo comes into contact with the gill filaments, it is surrounded and enclosed by leucocytes. Later these arrange themselves in two layers forming the brood pouch." He considers "the incubation of embryos in Cyclas as a case of ectoparasitism accompanied by the formation of a follicle at least in part, perhaps altogether mesodermal." Schereschewsky reviews Poyarkoff's work and can find no good evidence to substantiate it.

One fact may be significant to show that the glandular inner wall is not a structure which must be derived from other sources than filaments. In the dorsal part of the gills all of the filaments are fused to form a heavy-walled blood sinus. This wall is made up of cells which, in every respect, resemble those of the inner wall of the brood pouch.

NUTRITION OF THE EMBRYO. Schereschewsky observes that the embryo in the brood pouch is bathed by a distinct fluid which contains many acidophile granules. This fluid is the secretion of the large gland cells of the inner wall of the pouch.

Poyarkoff has a different theory. He says, "there are large cells of the inner wall of the brood pouch which serve for the nutrition of the embryo. At a certain time they become detached and fall into the lumen of the pouch. Their cytoplasm becomes homogeneous and eosinophile. Their nuclei take a uniform stain. The chromatin granules become almost completely indistinct. The embryo swallows these large cells. I have found these large shells in the intestine of some embryos. Stepanoff (1865) and Ziegler (1885) have noted this mode of nutrition." Poyarkoff further observes that the cells which have thus fallen into the cavity are replaced by leucocytes. Figure 9 is a copy of one of his illustrations. Schereschewsky has reviewed these observations and can find no evidence to justify them. In Calyculina and Sphaerium I have found undoubted evidence of secretion in the brood pouch. I have found a few cells thrown out into the lumen of the pouch but have considered this a normal phenomenon to be expected among actively secreting cells. As to the cells supposed to have been eaten by the embryo, may these not have been parasites?

SEXUAL MATURITY. Gross examination of the gills of Sphaerium revealed young so large that it was thought probable that these young might themselves be bearing embryos. Examination of microscopic sections proved that such a condition does not exist. The smallest specimen found to contain young was ten millimeters long. This is two millimeters longer than the largest young one found within the brood pouch. Several nine-millimeter specimens were sectioned but none were found to contain young. Young six, seven, and eight millimeters long have sex organs fully formed. I have no sections of very small Calyculina. The young of this form within the parent's gills are in the same stages of development as to the sex organs, as those of Sphaerium.

In the table which follows are included the results of gross examination of a number of Sphaeria. This is incomplete, since it was not possible to determine the presence of any young under five tenths of a millimeter. The animals examined were taken during July, 1913.

Size Length in mm.	*Total Examined*	*Total Bearing young*	*Percentage Bearing Young*
7	3	0	0
8	12	0	0
9	27	0	0
10	21	0	0
11	30	0	0
12	45	0	0
13	30	2	6.6
14	40	10	25
15	47	22	46.8
16	64	51	80
17	64	53	83
18	14	14	100
19	4	4	100

SPHAERIUM BEARING YOUNG OVER .5 MM. LONG.

Size	*Number bearing young*	*Total of class*	*Per cent of class*
13	2	30	6.6
14	10	40	25.
15	22	47	46.8
16	51	64	80.
17	53	64	83.
18	14	14	100.
19	4	4	100.

SIZE OF YOUNG TAKEN FROM ABOVE SPHAERIUM BY GROSS DISSECTION.

Size	*Frequency*	*Per cent of whole*
.5	1	.04
1.	16	6.
1.5	6	2.
2.	37	14.
2.5	9	3.
3.	26	10.
3.5	3	1.
4.	28	10.
5.	37	14.
6.	41	15.
7.	55	20.
8.	8	3.

267 young from 400 adults taken haphazard.

The size of the young was found to be independent of the size of the parent. An eight-millimeter young is as likely to be in a fourteen-millimeter parent as in one of eighteen millimeters length. The majority of the above contained two young, one in each inner gill. A few contained four.

Similar data were taken from Calyculina which were killed in June 1913.

Length of Parent	*Total Examined*	*Length of young*	*Number of young*
7	1	1.5	7
6	1	1.	4
8	1	1.5	9
7	1	1.5	16
7	1	1.5	10
8	1	2.	5
7	1	5.	3

The great variation in the number of young is probably due to the fact that some had already emerged from the parent pouch. I have no records which include totals of young of all stages. In one specimen I was able to count twenty-four.

No method has been discovered for determining the age of young or the period of incubation. I am inclined to believe that young in Calyculina are carried for one year or more. In a pond which was under observation for a period of over a year, adults were found in April to contain fully formed young. This pond had been dry from July of the year preceding until it became filled by melting snow in March. It had no inlet or outlet and received no overflow floods from any nearby ponds or streams. The number of young produced is probably ten to twenty in Calyculina and two to four in Sphaerium. These figures are based on the fact that these species during early spring contain about the above numbers of young, which when removed from the mother are able to take care of themselves.

AGE IN SPHAERIUM. The distinctness of growth-areas or rings in Sphaerium led to the belief that age might be determined by correlating size and number of rings. The entire surface of the shell (Pl. VI, Fig. 10) is thrown into narrow parallel ridges which represent growth-lines. At the beginning of a season these lines are widely separated; at the end they are crowded very closely together. One of these areas constitute a growth ring. In many specimens the several rings are further marked by slight differences of color due to deposits on the shell. The rings are usually very distinct though there are many forms in which they are very faintly differentiated.

The following table includes results obtained by measuring *Sphaerium simile*.

Total rings	*Number examined*	*Range of size in mm.*	*Average size in mm.*
1	46	5–10	8.6
2	58	9–14	11.2
3	86	9–17	13.2
4	116	12–18	16.9
5	67	12–19	17
6	20	12–19	17
7	6	16–19	17
8	1	18	18

While the above results are not conclusive they are certainly not altogether negative. The following facts seem significant.

1. No individuals attain a length of over 19 mm.

2. No individuals show more than 8 rings.

3. The averages of each class show an increase in length of about 2 mm. per ring.

4. The range of size included within each class is from 5 to 7 mm. This seems to indicate either that growth is very irregular in different individuals or that the rings do not indicate seasons. The fact that many specimens have very indistinct lines may account for some of these discrepancies. If one ring represents a year, the average age of a large specimen would be four or five years.

Regarding growth in the *Unionidae* Isely (1913) draws the following conclusions.

1. Rate of growth is exceedingly variable.

2. The summer months are the growth months.

3. Lines of arrested growth may be called rest rings, the conspicuous ones being usually winter rest rings. Occasionally the rest rings may be two or more years apart; more often, however, several equally prominent rings may be formed in one year. Prominent rest rings are generally due to double prismatic and epidermal layers.

Notes on Ecology. So far as observed the food consists mainly of diatoms. Many forms occur in ponds which become dry during the summer, remaining in that condition until the following spring. In aquaria I have observed that Calyculina will burrow down to the water level. Isely reports forms of Unionidae which were turned up by a plow in perfect condition.

The above observations emphasise the fact that very little is known about the habits of the forms considered.

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EXPLANATION OF PLATES IV–VI.

Plate IV. Figure 1. A diagrammatic dissection of Calyculina.

M —Mouth
Lp —Labial palps
Es —Esophagus
Lv —Liver
Sto —Stomach
Int —Intestine
R —Rectum
A —Anus
Cb G —Cerebral ganglion
Ps G —Parieto-splanchnic ganglion
P G —Pedal ganglion
K O —Kidney opening
T —Sperm follicles
O —Egg follicles
G D —Genital duct
G O —Genital opening
A A —Anterior adductor muscle
P A —Posterior adductor muscle
Ft —Foot
Cl Ch—Cloacal chamber
Ex S —Excurrent siphon

Sta	—Statocyst	*In S*	—Incurrent siphon
V	—Ventricle	*G*	—Gill
Au	—Auricle	*Man*	—Mantle
Pl	—Pericardium	*Shl*	—Shell
Kd	—Kidney		

Figure 2. Cross section of egg-bearing follicle of Calyculina.

O	—Egg	*Ep*	—Epithelium
Bt	—Basement membrane	*Yo*	—Young egg

Figure 3. Cross section of sperm-bearing follicle.

Sp Mc	—sperm mother cells.	*Sp*	—sperm

Figure 8. Frontal section of inner gill of Calyculina showing brood pouch. Drawn with Edinger machine.

O L	—Outer lamella	*Ct*	—Cross threads
I L	—Inner lamella	*Em*	—Embryo
Ci	—Cilia	*Sh*	—Shell remains
G Fil	—Gill filament	*Cl Ch*	—Cloacal chamber
Bs	—Blood space	*Gc*	—Gland cells of inner wall.
I W	—Inner wall of brood pouch	*Sec*	—Secretion
O W	—Outer wall of brood pouch		

Plate V. Figure 4. Diagrammatic section of sex organs. *Calyculina.*

T	—Sperm follicles	*Gd*	—Genital duct
Or	—Egg follicle	*G O*	—Genital opening.

Figure 5. Diagrammatic cross section through the region of the reproductive organs. *Calyculina.*

Pp	—Pericardium	*M*	—Mantle
V	—Ventricle	*Ft*	—Foot
Au	—Auricle	*Cl Ch*	—Cloacal chamber
Bs	—Blood space	*O*	—Egg follicle
R	—Rectum	*T*	—Sperm follicles
I G	—Inner Gill	*Nc*	—Nerve cords
O G	—Outer Gill	*C T*	—Connective tissue

Figure 6. Diagrammatic cross section of Calyculina to show opening of genital ducts.

G O —Genital opening
N C —Nerve cord
Cl Ch —Cloacal chamber
I G —Inner gill
O G —Outer gill
Ft —Foot
P —Pericardium
K —Kidney
R —Rectum
Blv —Posterior aorta
M —Mantle

Figure 7. Frontal section of neutral part of inner gill, showing fusion of the two lamellae. *Calyculina.*

I L —Inner lamella
O L —Outer lamella
Bls —Blood space
Ifj —Interfilamentary junctions
Ct —Cross threads

Figure 9. Part of inner wall of the brood pouch of Cyclas. (Copied from Poyarkoff).

L —Leucocyte lodged at base of cells
F —Leucocyte just entering wall of pouch
A —Leucocyte beginning to divide
N —Polynucleate cell

Plate VI. Figure 10. Shell of Spaerium simile showing growth lines.

U —Umbo
1, 2, 3, 4, —Growth lines

Figure 11. Diagrammatic reconstruction of a portion of the inner gill of Calyculina, showing the relation of brood pouches to gill filaments.

I L —Inner lamella
O L —Outer lamella
Cl Ch —Cloacal chamber
If J —Interfilamentary junction
G F —Gill filament
Em —Embryo
I W —Inner wall of brood pouch
O W —Outer wall of brood pouch
Bls —Blood space